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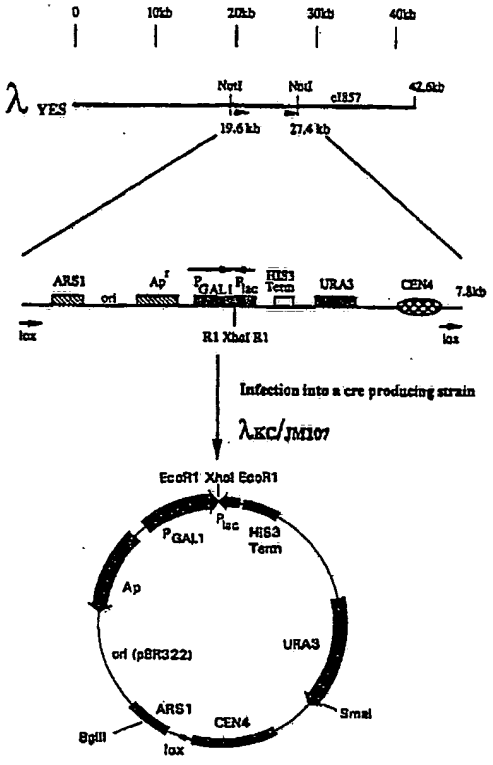
(54) Title: GENETICALLY ENGINEERED EUKARYOTIC ORGANISM CAPABLE OF DETECTING THE EXPRESSION OF HETEROLOGOUS ION CHANNELS

(57) Abstract

This invention relates to genetically engineered eukaryotic organisms, such as yeast, that are made capable of detecting the expression of heterologous ion channels. These organisms include a potassium transport defective phenotype eukaryotic organism transformed with DNA that suppresses the potassium transport defective phenotype in the organism. A potassium transport gene is set out in Sequence Id. No. 1. This genetically engineered organism can be used to screen for new herbicides or drugs.



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"Genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels".

Background of the Invention

This application is a continuation-in-part of U.S. Serial No. 874,846 filed April 27, 1992. This invention was made with government support under grant No. DCB8711346 awarded by National Science Foundation and grant No. 90-37261-5411 awarded by U.S. Dept. of Agriculture. The government has certain rights in the invention.

Field of the Invention: This invention relates to genetically engineered eukaryotic organisms, such as yeast, that are made capable of detecting the expression of heterologous ion channels. This genetically engineered organism can be used to screen for new herbicides or pharmaceuticals.

Description of Prior Work in the Field

Advances in molecular biology have provided the means to transform organisms to contain foreign genes. Such genes can be transformed into the organism to affect its function. B. Lewin, Genes, 300-333 and 589-631 (1983) (hereby incorporated by reference).

In plants and fungi the uptake and intracellular concentration of potassium serve a variety of vital functions including the control of cell shape and turgor, the establishment of an ionic milieu compatible with enzyme function, and the enhancement of plasma membrane proton pump function. Serrano, R.

Plasma Membrane ATPase of Fungi and Plants as a Novel Type of Proton Pump; Curr. Top. Cell. Regul. 23:87-126 (1984).

The inventor and other colleagues from Northwestern University have cloned two genes TRK1 and TRK2 that encode potassium transporters in Saccharomyces cerevisiae. The TRK2 gene encodes a low-affinity and the TRK1 gene encodes for a high-affinity potassium transporter. Cells deleted for both TRK1 and TRK2 are hypersensitive to low pH. They are also severely limited in their ability to take up potassium. Ko et al. TRK1 and TRK2 Encode Structurally Related Potassium Transporter in Saccharomyces cerevisiae, Molec. and Cell. Bio. 11:4266-4273 (Aug. 1991) (hereby incorporated by reference); Ko et al. TRK2 is Required for Low Affinity K^+ Transport in Saccharomyces Cerevisiae, Genetics 125:305-312 (June 1990) (hereby incorporated by reference).

Herbicide and drug identification frequently involves the detection of single compounds that show potential as plant growth inhibitors and/or pharmaceuticals from large numbers of naturally occurring and synthetic substances. A disadvantage of current identification processes is that they can be time consuming and expensive. Also, not all commonly used screening procedures demonstrate a specific mode of action of the active compounds.

The present invention relates to method for drug and herbicide testing that may significantly reduce assay time and cost. A unique feature of this method is its capacity to reveal the specific molecular system affected by the assayed compounds.

Summary of the Invention

The invention consists of a microbial system that can serve as a sceen for compounds that inhibit potassium channels. Specifically, a genetically engineered strain of yeast, rendered defective in potassium uptake and supplied with a plant gene has been developed by the inventor. The altered yeast strain has been shown to be effective as a cell culture system for screening potassium channel-inhibiting compounds.

More specifically, the present invention provides a genetically engineered organism that includes a potassium transport defective phenotype. More specifically, this invention provides a genetically engineered yeast strain (ATCC No. 74144) that is deleted for both of its endogenous potassium transporters (TRK1 and TRK2) and which includes a heterologous plant potassium channel gene from Arabidopsis thaliana. This new strain of yeast allows for rapid screening of chemical compounds for anti-potassium channel activity by measuring a compound's ability to inhibit the growth of the genetically engineered yeast cells. These compounds may have activity as a herbicide or pharmaceutical.

Additionally, this invention provides cDNA for a potassium channel gene. This cDNA sequence is shown in Sequence Id. No. 1 and is incorporated into a plasmid identified as ATCC No. 75224. This gene can be used to make a genetically engineered eukaryotic organism capable of detecting heterologous ion channels. This organism can be made by transfecting a potassium transport defective phenotypic organism with DNA that suppresses the potassium transport defective phenotype in the organism.

More specifically, this invention provides a genetically engineered eukaryotic organism dependent on a heterologous ion channel for growth. The organism has the characteristics of the strain deposited as ATCC No. 74144. This organism is a Saccharomyces cerevisiae deleted for TRK2 and TRK1, or only TRK1, that is transfected with the DNA sequence set out in the Sequence Id. No. 1.

Still, additionally, this invention provides a method to screen compounds for their ability to inhibit potassium transport in vivo. This screening method involves: adding the compound to be screened to a genetically engineered organism capable of detecting heterologous ion channel wherein the organism is a potassium transport defective phenotypic organism transformed with DNA that suppresses the potassium transport defective phenotype in the organism, to a media containing potassium, and determining whether the organism's growth is inhibited.

Brief Description of the Figures

Figure 1 shows TEA and BA^{2+} inhibition of KAT1 in vivo. Approximately 10^5 CY162/pKAT1 cells were plated on to GAL-URA 0.2K solid media. 20 µl of 1 M TEA and 20µl of 1 M $BaCl_2$ were applied to sterile filter disks placed on the media; a halo of inhibited cell growth can be seen around the filters on the 0.2 mM K^+ containing plate. $BaCl_2$ precipitated out of the medium in the region surrounding the Ba^{2+} filter disk.

Figure 2 shows that barium and TEA do not inhibit the growth of CY162/pKAT1 cells when these cells are grown in the presence of a high concentration of potassium.

Figure 3 shows a plasmid into which the KAT1 cDNA was cloned during the construction of the library.

Figure 4 shows the arabidopsis cDNA expression vector.

Detailed Description of the Invention and Best Mode

The present invention provides a genetically engineered organism capable of detecting heterologous ion channels and a method to use the same in screening for new herbicide or drug compounds. The term "heterologous" in this context means the expression in Saccharomyces cerevisiae of any nonSaccharomyces cerevisiae gene, i.e. any ion channel gene from another organism. More specifically, this invention provides a yeast strain deleted for both of its endogenous potassium transporters TRK1 and TRK2 and that includes the newly discovered heterologous plant potassium channel gene (Sequence Id. No. 1) from Arabidopsis thaliana deposited with the ATCC under the Budapest Convention and that has received ATCC number 74144. It should be noted, however, that a genetically engineered yeast stain deleted for TRK1 and including the plant potassium channel gene from Arabidopsis thaliana is also contemplated by this invention. The altered yeast strain has been shown to be effective as a cell culture system for screening potassium channel-inhibiting compounds. The system is easily adaptable to microtiter plate technology rendering the method rapid and inexpensive. Cell growth and inhibition as determined by turbidity, can be measured by standard spectrophotometric instrumentation. The identification of compounds that both completely and partially inhibit potassium channel activity is also possible. The present yeast

strain containing the plant gene is useful for screening pharmaceuticals because all potassium channels are sensitive to the same compounds; thus, if a compound was identified as having a potassium channel inhibiting activity it could be further tested for likely pharmaceutical utility.

In the present invention, the endogenous TRK1 (or TRK1 and TRK2) transporters are deleted in order to 1) detect function of the heterologous ion channel and 2) to make the strain dependent on the heterologous channel for growth. It should be noted, however, that one would not need to delete TRK1 (or TRK1 and TRK2) in order to make the S. cerevisiae cells dependent on the heterologous potassium channels for growth. One could simply isolate uncharacterized mutations in these that have the effect of significantly reducing their function. As such these organisms include a potassium transport defective phenotype transformed with DNA that suppresses the potassium transport defective phenotype in the organism.

It should be noted, however, that other potassium-transporting proteins, (not just those known to function as channels) could also suppress the potassium transport defect in trk1Δ trk2Δ cells since these proteins could also represent essential plant proteins and thus, be useful in the screening process for new herbicides or drugs.

The KAT1 gene has the following characteristics: 1) KAT1 suppresses the Trk- phenotype of S. cerevisiae cells deleted for their endogenous potassium transporters; 2) the inferred protein sequence includes a cluster of six putative membrane-spanning domains and conserved amino acids sequences corresponding to the presumptive voltage-sensing (S4)

and pore-forming (SS1-SS2 or H5) regions; and 3) potassium channel-specific blockers (TEA and Ba^{2+}) inhibit of KAT1 in vivo. Alternatively, Schizosaccharomyces pombe, could be transformed with pKAT1 due to the presence of the Saccharomyces cerevisiae selectable marker URA3.

The gene that encodes for the plant potassium gene was identified as follows:

1. Media and Strains. YNB and LS media were prepared as described by Sherman et al. and Gaber etal (Sherman, F., Fink, G.R. and Hicks, J. (1986) (hereby incorporated by reference) Methods in Yeast Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Gaber, R.F., Styles, C.A. and Fink, G.R. (1988) Mol. Cell. Biol. 8, 2848-2859). AA-URA is medium supplemented with all amino acids and nucleosides except uracil. Media with galactose or glucose as the sole carbon source are indicated as GAL and GLU. Ko and Gaber describe construction of the S. cerevisiae strain, CY162, MATα ura3-52 trk1Δ his3 200 his4-15 trk2Δ 1::pCK64 (Ko, C.H. and Gaber, R.F. (1991) Mol. Cell. Biol. 11: 4266-4273). Yeast transformation was performed by electroporation (Becker, D.M. and Guarente, L. (1991) Meth. Enzym. 194, 182-187). Plasmids were selected and propagated in E. coli strain HB101 on Luria broth (LB) medium supplemental with 50μg/ml ampicillin. LB medium is used to grow E. Coli. LB medium and ampicillin is used to maintain selection for the presence of ampicillin resistance-conferring plasmids in E. Coli like pKAT1.

2. cDNA Cloning. The Arabidopsis thaliana cDNA library was constructed in the λYES yeast/E. coli shuttle vector. (Stanford University, Stanford, CA) (Elledge, S.J., Mulligan, J.T., Ranier, S.W.,

Spottswood, M. and Davis, R.W. (1990) Proc. Natl. Acad. Sci. USA 88, 1731-1735) (hereby incorporated by reference). See Figures 3 and 4. This library was made from mRNA extracted from leaves, shoots, stems and flowers of plants at all stages of development (Elledge et al., infra.).

The λYes vector is a multifunctional vector. It is capable of replicating as a lambda phage, a plasmid lysogen in E. coli. or as a centromere plasmid in yeast. The plasmid part of the vector can be automatically looped out of the lambda phage by site specific recombination using the cre protein and lox sites in the vector (Sternberg, et al. 1983. In Mechanisms of DNA Replication and Recombination. UCLA Symposia on Molecular and Cellular Biology. Vol. 10, pp. 671-684; Sauer and Henderson. 1988. Gene 70; 331-41). The cDNAs are inserted nondirectionally. In one direction, they can be expressed from the E. coli lac promoter, with a ribosome binding site and an ATG between the promoter a cloning site. In the other direction, they can be expressed from the yeast gall promoter, and are followed by a yeast transcription termination site. A rough map of the vector is shown on the next page. The selectable markers are Amp resistance in E. coli and URA3 in yeast.

The cDNAs are inserted into an XhoI site flanked by EcoRI sites. In theory, the XhoI sites should be regenerated during the cloning. Occasionally, an XhoI site may be missing and the insert must be excised with EcoRI.

Library: PolyA purified mRNA was prepared from the above-ground parts of Arabidopsis plants which varied in size from those which had just opened their

primary leaves to plants which had bolted and were flowering. A library of 10 million independent recombinants was amplified as lytic phages on plates. Approximately 90-95% of the clones in the amplified library contain inserts, and the titer is 6.2×10^9 per ml, a typical titer for this vector.

Storage. The amplified phage stock is in LB with 7% DMSO. The lambda is stable for several weeks at room temperature.

Propagation and Screening. The phage backbone is lambda gt6. It can be treated as a standard lambda phage (e.g. grow lytically on LE392 pMC9 to amplify, or on LE392 to screen for expressed proteins). Alternatively, it can lysogenize as an amp resistant plasmid in a strain which expresses the lambda repressor, cI.

Plasmid Recovery. The plasmid part of the vector can be looped out of the lambda by infecting a strain which expresses cre and cI (eg. BNN132 [JM107 lysogenized with λ KC]). Plasmid DNA can be prepared by:

1) Grow BNN132 overnight in LB + maltose (.2%) + kanamycin (50μ/ml).
2) Spin down and resuspend in lambda dil (5M) buffer.
3) Add phage library to an m.o.i, of .01, incubate 20 minutes at 37°C.
4) Grow nonselectively for 30 minutes in LB.
5) Plate on LB + ampicillin, grow overnight at 37°C.
6) Scrape plates and do a standard plasmid preparation.

Yeast. The yeast transformation protocol of Burgers and Percival (Analytical Biochemistry 163:391-397) (hereby incorporated by reference).

The library starts out as lambda phage library. Upon induction of the phage (being grown in E. coli) the plasmid "pops out" and can then be amplified and harvested from the E. coli culture. The KAT1 cDNA is a XhoI fragment inserted at the XhoI site in the vector. Thus, the size of pKAT1 is the vector (7.80 kb) plus the cDNA insert (about 2.24 kb) + 10 kb. The EcoR1 XhoI EcoR1 is the "poly-cloning" site. The KAT1 cDNA fragment is inserted into the XhoI site here. The following sequences are contained on the plasmid:

1. P_{GAL1}: Promoter region found the yeast GAL1 gene; this sequence promotes expression of the cDNA insert upon growth on galactose as the sole carbon source.
2. P_{lac}: promoter from the E. coli lac operon; used for conditional expression of cDNAs (cloned in the opposite direction) in E. coli.
3. HIS3 Term: sequence containing the transcriptional termination signals from the yeast HIS3 gene; used to ensure termination of the cDNA insert when expressed in yeast.
4. URA3: the yeast URA3 gene; used as the selectable marker to select for presence of the plasmid in ura3 yeast recipient strains.
5. CEN4: sequences encoding the yeast centromere (#IV); used to maintain stability of the plasmid during yeast cell division, i.e., this makes the plasmid function as a "mini-chromosome".
6. ARS1: this is an "autonomously replicating sequence" that serves as a site of initiation of replication in yeast.
7. ori (pBR322): this is the site of replication initiation that is used when the plasmid is

propagated in an E. coli host.

8. Ap^r: the ampicillin resistance gene that allows selection for the plasmid in E. coli.

Expression of the cloned inserts are under control of the inducible GAL1 promoter. The library was introduced into CY162 cells by transformation. Initial selection and subsequent screening of the transformants were carried out on AA-URA to maintain selection for the plasmids. Ura^+ transformants were selected on glucose-containing medium supplemented with 100 mM potassium (GLU-URA 100K) and replicaplated to GAL-URA 100K to induce expression of the cloned cDNAs. Following an overnight incubation the transformants were replica plated to GAL-URA containing 7 mM potassium (7K) to identify cDNAs able to confer suppression of the potassium transport-defective phenotype (Trk-) of the recipient cells.

3. DNA Sequencing. Dideoxy sequencing of pKAT1 was performed using SEQUENASE (U.S. Biochemicals) (Sanger, F. et al. Proc. Natl. Acad. Sci. USA 74:5463-67 (1977) (hereby incorporated by reference). Double stranded template DNA was sequenced using specific oligonucleotide primers synthesized at the Northwestern University Biotechnology Facility.

Primers used for sequencing (all represented in 5' to 3' direction of polarity:

For sequencing the noncoding strand:

GAL1.	TACTTTAACGTCAAGGAG	Sequence Id No. 2
GAL2.	CTAAGCTCCGCAAACAC	Sequence Id No. 3
GAL3.	CTTCTAGTTGACAGTC	Sequence Id No. 4
GAL4.	CGGAAGCGAACTAGG	Sequence Id No. 5
GAL5.	CATTGTGCTGGATGT	Sequence Id No. 6
GAL6.	GATGTTCAACCTCGG	Sequence Id No. 7
LAC5inv.	TACTGCGGATAAGCA	Sequence Id No. 8
LAC4inv.	GGATGGGAAGAGTGG	Sequence Id No. 9
LAC3inv.	TAGTGAAACCGCTGG	Sequence Id No. 10
LAC2inv.	ATCCATAGAAGAGCT	Sequence Id No. 11
LAC1inv.	GCATGTATATCTGCA	Sequence Id No. 12

For sequencing the coding strand:

GAL2inv.	GCTGAGTAAATAACT	Sequence Id No. 13
GAL3inv.	ATTCGTATTTTCTTA	Sequence Id No. 14
GAL4inv.	TCAAGCCTTGCAAAT	Sequence Id No. 15
GAL5inv.	TGCTTCTTTGAAATT	Sequence Id No. 16
GAL6inv.	AGGTTGGTCATATTTCCAA	Sequence Id No. 17
LAC5	TTGTTCTTACTGTGA	Sequence Id No. 18
LAC4	GTCGGAAGTCGGATTCG	Sequence Id No. 19
LAC3	GGTTGCTTGAGCTGC	Sequence Id No. 20
LAC2	ACCATCCCAAATGACAT	Sequence Id No. 21
LAC1	TGTGGAATTGTGAGCGG	Sequence Id No. 22

DNA sequence analysis was done using the DNA inspector IIe (Textco, Lebanon, NH) and the Genetics Computer Group (GCG, Madison, Wisconsin) software.

4. Southern Blot Analysis. Two µg of genomic DNA extracted from A. thaliana (Columbia ecotype) was digested with EcoRl, electrophoresed on 0.8% agarose and transferred to nylon membrane. The KAT1 probe

was prepared by random hexamer [α-32_p] dCTP labelling of the 2.2-kb XhoI insert contained in pKAT. Hybridization overnight at 65°C was followed by three washes at 60° for 15 minutes each in 6X standard saline citrate/0.1% SDS (Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6).

RESULTS

An Arabidopsis thaliana cDNA library was screened for sequences that suppress the potassium transport defect (Trk- phenotype) of trk1 Δ trk2 Δ cells (CY162) by conferring growth on potassium-limiting medium. From approximately 40,000 Ura^+ transformants, a single clone was obtained that allows growth of CY162 cells on 7 mM potassium, galactose-containing medium (GAL 7K). The cloned plasmid, pKAT1, was recovered by transformation of E. coli and reintroduced into CY162 by transformation. This plasmid is assigned ATCC No. 75224. All Ura^+ transformants containing pKAT1 were able to grow on GAL 7K.

Southern analysis using the cDNA insert contained in pKAT1 as a probe revealed the presence of homologous sequences in the A. thaliana genome.

1. KAT1 Completely Suppresses the Trk- Phenotype of trk1 Δ trk2 Δ Cells. Wild type (TRK1 and TRK2) S. cerevisiae cells are able to grow on media supplemented with 0.2 mM potassium chloride (0.2K);(Gaber, R.F., Styles, C.A. and Fink, G.R. (1988) Mol. Cell. Biol. 8, 2848-2859). To determine the level of KAT1 suppression, colonies of CY162 cells containing pKAT1 were replica plated to GAL 0.2K. pKAT1 conferred growth on GAL 0.2K but not on GLU 0.2K, consistent with the conditional expression of

the cDNA. Growth of CY162/pKATL cells on GAL 0.2K was indistinguishable from that of wild-type cells.

2. DNA sequence Analysis Suggests KAT1 Encodes a Potassium Channel. The cDNA sequence in pKAT1 revealed an open reading frame of 2,031 nucleotides capable of encoding a protein of 677 amino acids (78 kD, Sequence Id. No.1). Northern blot analysis, using KAT1 sequences as a probe, detected a 2.2-kb message that was present in very low abundance, indicating that pKAT1 contains a full-length or near full-length cDNA.

EXAMPLE 1
Tetraethylammonium and Ba^{2+} Inhibit KAT1 Function *in vivo*

Although the ability to suppress the potassium transport deficient phenotype of *trk1Δ trk2Δ* cells and the structural features inferred from the KAT1 DNA sequence suggested that KAT1 is potassium channel, the inventor further tested this interpretation by testing the effect of tetraethylammonium and Ba^{2+} ions on the function of KAT1. Tetraethylammonium (TEA) and Ba^{2+} are specific inhibitors of many voltage-gated potassium channels and appear to block channel conductance by interacting with sites normally occupied by potassium ion (Hille, B. (1981) Ionic Channels of Excitable Membranes. Sunderland, MA, Sinauer; Mackinnon, R. And Yellen, G. (1990) Science 250, 276-278; Yellen, G., Jurman, M.E., Abramson, T. and MacKinnon, R. (1991) Science 251, 939-941). This has been further supported by recent experiments in which mutations residing in the region thought to constitute the lining of the channel pore were shown to affect the binding of tetraethylammonium.

If KAT1 is a potassium channel, growth of CY162/pKAT1 cells on potassium limited medium would be inhibited by TEA and Ba^{2+}. Tetraethylammonium and Ba^{2+} were applied to filter displaced on to lawns of CY162/pKAT1 cells growing on GAL 100K and GAL 0.2K solid media (See Figs. 1 and 2). Growth of CY162/pKAT1 cells was inhibited by tetraethylammonium and Ba^{2+} on 0.2 mM, but not on 100 mM potassium. Low concentrations of potassium should be added to the screening media. The expression of KAT1 will allow trk1 trk2 cells to grow on very low concentrations of potassium (as low as wild-type TRK1 TRK2 cells). Therefore using a concentration of approximately 0.1 to 0.2 mM potassium would allow even slight inhibition of KAT1 to result in the inhibition of growth of the CY162/pKAT1 cells. In contrast, similar tests using CY162 cells containing TRK1 carried on a centromeric plasmid (pRG295-1) showed no inhibition by these compounds (Gaber, R.F., Styles, C.A. and Fink, G.R. (1988) Mol. Cell. Biol. 8, 2848-2859).

The isolation of KAT1 indicates that S. cerevisiae can be used as a powerful and convenient method of isolating potassium channel cDNAs from higher eukaryotes. Other libraries are being screened to determine whether cDNAs encoding human potassium channels can also be isolated using this system. Additionally, the genetically engineered organism of this invention can be used to detect organism the expression of heterologous ion channels. This can be used to screen compounds as potential new herbicides or drugs, as shown in Example 1.

Although the invention has been described primarily in connection with special and preferred embodiments, it will be understood that it is capable of modification without departing from the scope of

the invention. The following claims are intended to cover all variations, uses, or adaptations of the invention, following, in general, the principles thereof and including such departures from the present disclosure as come within known or customary practice in the field to which the invention pertains, or as are obvious to persons skilled in the field.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: GABER, RICHARD F.

(ii) TITLE OF INVENTION: GENETICALLY ENGINEERED EUKARYOTIC ORGANISM CAPABLE OF DETECTING THE EXPRESSION OF HETEROLOGOUS ION CHANNELS AND METHOD TO USE SAME

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
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(D) STATE: ILLINOIS
(E) COUNTRY: USA
(F) ZIP: 60606-4002

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
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(B) REGISTRATION NUMBER: 31,327
(C) REFERENCE/DOCKET NUMBER: NU-9211CIP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312/456-8000

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2173 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATAATCTCTC CTTTTAATCC ACGTTACAGA GCGTGGGAGA TGTGGCTAGT
ATTACTAGTT 240

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GAAAATAGCA 420

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CCAAGAGAAA TGCTTTTTGA CATTTTCTTC ATGATGTTCA ACCTCGGTTT
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TACTATAGAC CACAACCATT CACAGTAAGA ACAACCGAGC TATCTCAAAT
ACTGCGGATA 1440

AGCAGAACAT CGCTGATGAG TGCGATGCAT GCTCATGCTG ACGATGGACG
AGTCATCATG 1500

AACAATCTCT TCATGAAACT TAGAGGGCAA CAGTCAATAG CAATAGATGA
TTCGAATACT 1560

AGTGGTCACG AAAACAGAGA TTTCAAAAGC ATGGGATGGG AAGAGTGGAG
AGATTCAAGA 1620

AAAGATGGCT ATGGTTTAGA TGTTACGAAT CCGACTTCCG ACACTGCTCT
AATGGATGCG 1680

ATTCACAAGG AAGATACTGA AATGGTTAAG AAGATACTTA AGGAACAAAA
GATAGAGAGA 1740

GCCAAAGTGG AAAGATCAAG TAGTGAAACC GCTGGAAGAA GTTACGCTAA
CGATTCATCG 1800

AAAAAAGATC CATATTGCAG CTCAAGCAAC CAAATCATCA AGCCATGCAA
ACGAGAAGAA 1860

AAGAGAGTTA CCATCCACAT GATGTCTGAG AGCAAGAACG GGAAGTTGAT
ACTCTTACCA 1920

TCATCCATAG AAGAGCTTCT AAGACTTGCA AGTGAGAAGT TTGGAGGCTG
CAACTTCACA 1980

AAGATCACCA ATGCGGACAA CGCTGAGATT GATGATTTAG ATGTCATTTG
GGATGGTGAT 2040

CATTTGTATT TTTCATCAAA TTGAGTTTGA AAACTCGACT TCATTTATAG
AGCATGTATA 2100

TCTGCAGATA ATGTATTTTT ACCCGGTTTC ATAGAAAAGT CTAGATTATC
CCCTGACGTA 2160

GCTCGAGGAA TTC 2173

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TACTTTAACG TCAAGGAG
18

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CTAAGCTCCG CAAACAC
17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTTCTAGTTG ACAGTC
16

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGAAGCGAA CTAGG
15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CATTGTGCTG GATGT
15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATGTTCAAC CTCGG
15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TACTGCGGAT AAGCA
15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GGATGGGAAG AGTGG
15

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TAGTGAAACC GCTGG
15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATCCATAGAA GAGCT
15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

GCATGTATAT CTGCA
15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GCTGAGTAAA TAACT
15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATTCGTATTT TCTTA
15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

TCAAGCCTTG CAAAT
15

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGCTTCTTTG AAATT
15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGGTTGGTCA TATTTCCAA
19

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

TTGTTCTTAC TGTGA
15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTCGGAAGTC GGATTCG
17

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GGTTGCTTGA GCTGC
15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

ACCATCCCAA ATGACAT
17

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGTGGAATTG TGAGCGG
17

I Claim:

1. A composition comprising: cDNA for a plant potassium channel.

2. The composition of Claim 1 wherein the DNA sequence of said cDNA consists essentially of the DNA sequence of Sequence Id. No. 1.

3. A composition comprising the cDNA contained in plasmid deposited as ATCC No. 75224.

4. A genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels comprising: a potassium transport defective phenotypic eukaryotic organism transformed with DNA that suppresses potassium transport defective phenotype in said organism.

5. The composition of Claim 4 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

6. The composition of Claim 4 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK1.

7. The composition of Claim 5 wherein said DNA consists essentially of the DNA sequence set out in Sequence Id. No. 1.

8. The composition of Claim 6 wherein said DNA consists essentially of the DNA sequence set out in Sequence Id. No. 1.

9. A genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels having the characteristics of the organism deposited as ATCC No. 74144.

10. A genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels made by the process comprising:

a. deleting or mutating genes from a eukaryotic

organism to render it potassium transport defective; and

b. transforming said organism to add a gene encoding a potassium channel.

11. The genetically engineered organism of Claim 10 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

12. The genetically engineered organism of Claim 10 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK1.

13. The genetically engineered organism of Claim 11 wherein said gene encoding a potassium channel consists essentially of the DNA sequence set out in Sequence Id. No. 1.

14. A method to make a genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels comprising the process of:

a. deleting or mutating genes from a eukaryotic organism to render it potassium transport defective; and

b. transforming said organism to add a heterologous gene encoding for a potassium channel.

15. The method of Claim 14 wherein said organism Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

16. The method of Claim 14 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK1

17. The method of Claim 14 wherein said gene encoding a potassium channel consists essentially of the DNA sequence set out in Sequence Id. No. 1.

18. A method to screen a compound for the ability to inhibit potassium transport in an organism

comprising:

a. adding said compound to a genetically engineered eukaryotic organism capable of detecting the expression of heterologous ion channels comprising a potassium transport defective phenotypic eukaryotic organism transformed with DNA that suppresses the potassium transport defective phenotype in said organism, to a media containing potassium;

b. determining whether said compound inhibits growth of said organism.

19. The method of Claim 18 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1 and the DNA consists essentially of the DNA sequence set out in Sequence Id. No. 1.

20. The method of Claim 18 wherein said organism is Saccharomyces cerevisiae deleted or mutated for TRK1 and the DNA consists essentially of the DNA sequence set out in Sequence Id. No. 1.

AMENDED CLAIMS

[received by the International Bureau on 1 October 1993 (01.10.93); original claims 18-20 cancelled; original claims 1-16 amended; other claims unchanged (3 pages)]

1. A composition consisting of: a cDNA that encodes a plant potassium channel.

2. The composition of Claim 1 wherein the DNA sequence of said cDNA consists of the DNA sequence of Sequence Id. No. 1.

3. A composition consisting of: the cDNA contained in a plasmid deposited as ATCC No. 75224.

4. A genetically engineered strain of yeast containing a heterologous ion channel comprising: a potassium transport defective phenotypic strain of yeast transformed with DNA that suppresses potassium transport defective phenotype in said yeast.

5. The genetically engineered strain of yeast of Claim 4 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

6. The genetically engineered strain of yeast of Claim 4 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK1.

7. The genetically engineered strain of yeast of Claim 4 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK1.

8. The genetically engineered strain of yeast of Claim 6 wherein said DNA consists essentially of the DNA sequence set out in Sequence Id. No. 1.

9. A genetically engineered strain of yeast

9. A genetically engineered strain of yeast containing a heterologous ion channel deposited as ATCC No. 74144.

10. A genetically engineered strain of yeast containing a heterologous ion channel made by the process comprising:

a. deleting or mutating genes from a strain of yeast to render it potassium transport defective; and

b. transforming said yeast to add a gene encoding a potassium channel.

11. The genetically engineered strain of yeast of Claim 10 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

12. The genetically engineered strain of yeast of Claim 10 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK1.

13. The genetically engineered strain of yeast of Claim 11 wherein said gene encoding a potassium channel consists essentially of the DNA sequence set out in Sequence Id. No. 1.

14. A method to make a genetically engineered strain of yeast containing a heterologous ion channel comprising the steps of:

a. deleting or mutating genes from a strain of yeast to render it potassium transport defective; and

b. transforming said strain of yeast to add a

heterologous gene encoding for a potassium channel.

15. The method of Claim 14 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK2 and TRK1.

16. The method of Claim 14 wherein said yeast is Saccharomyces cerevisiae deleted or mutated for TRK1.

STATEMENT UNDER ARTICLE 19

The amendment of claims 1-16 is made in order to more clearly distinguish these claims from Ko et al., "TRK2 is Required for Low Affinity K^+ Transport in Saccharomyces cerevisiae," pages 305-312 in Genetics, Volumne 125, issued June 1990, Chandy et al., WO, A, 92/02634 20 February 1992, Ko et al., "TRK1 and TRK2 Encode Structurally Related K^+ Transporters in Saccharomyces cerevisiae, pages 4266-4273 in Molecular and Cellular BIology, Volume 11, Number 8, issued August 1991. All of these documents were cited in the international search report as being of particular relevance to the claimed invention. Full support for the amendments to these claims can be found on pages 3, 6 and 14.

Fig. 1

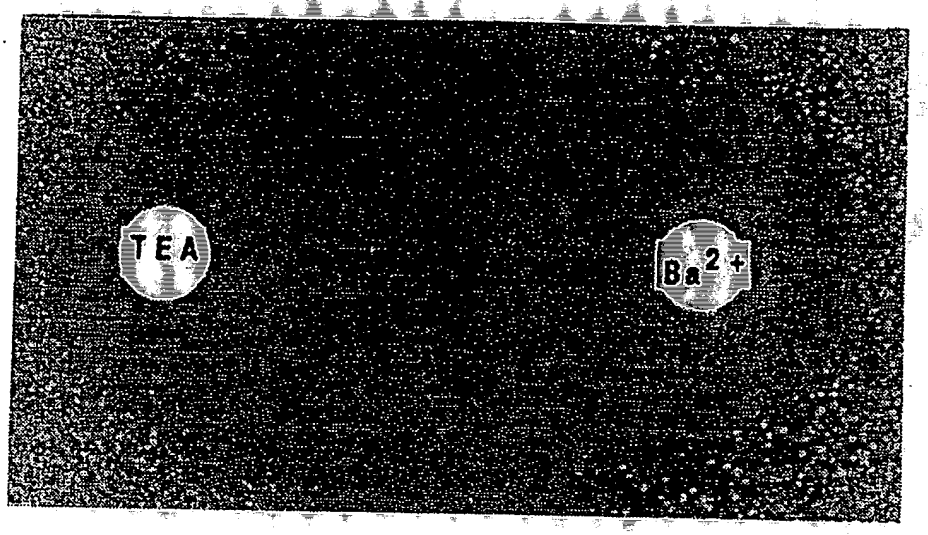


Fig. 2

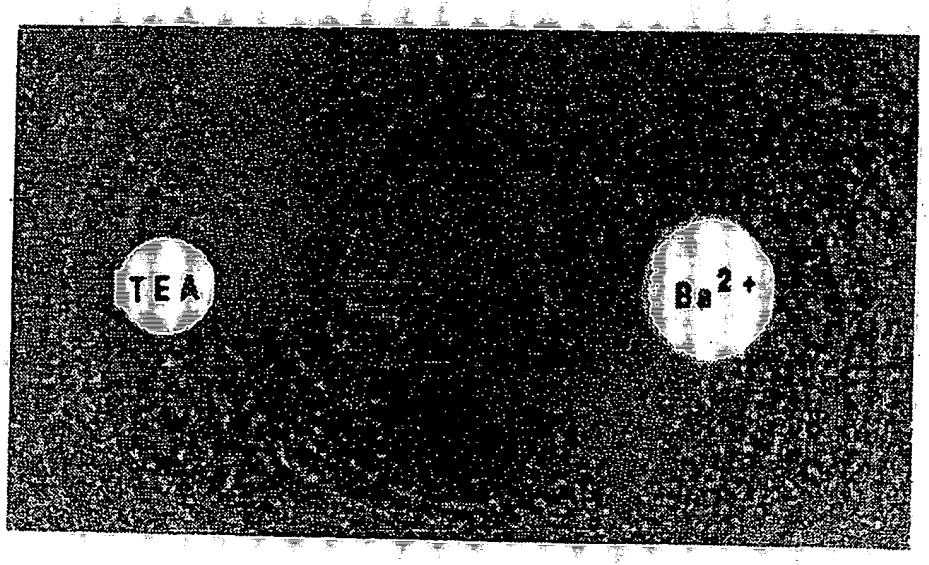


Fig. 3

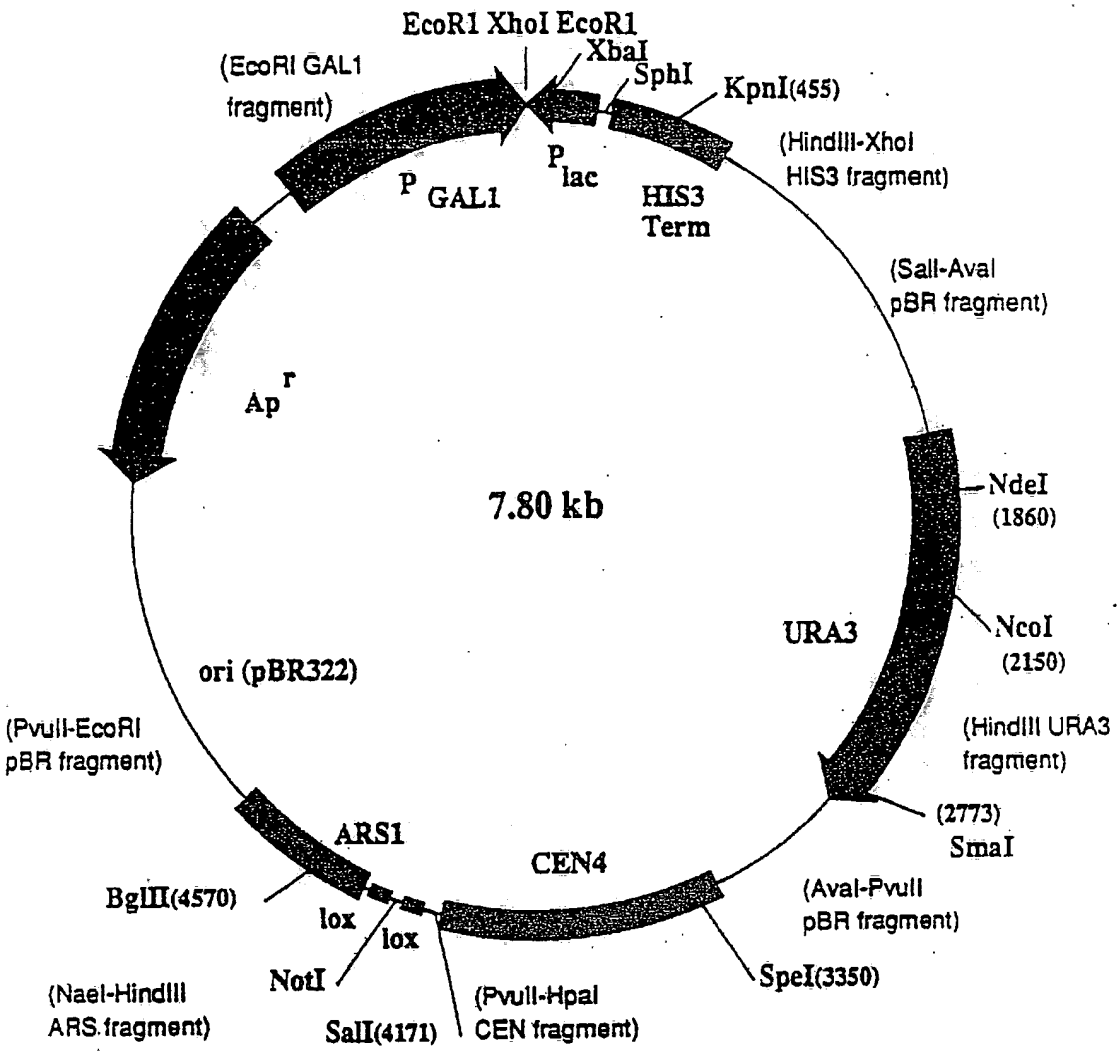
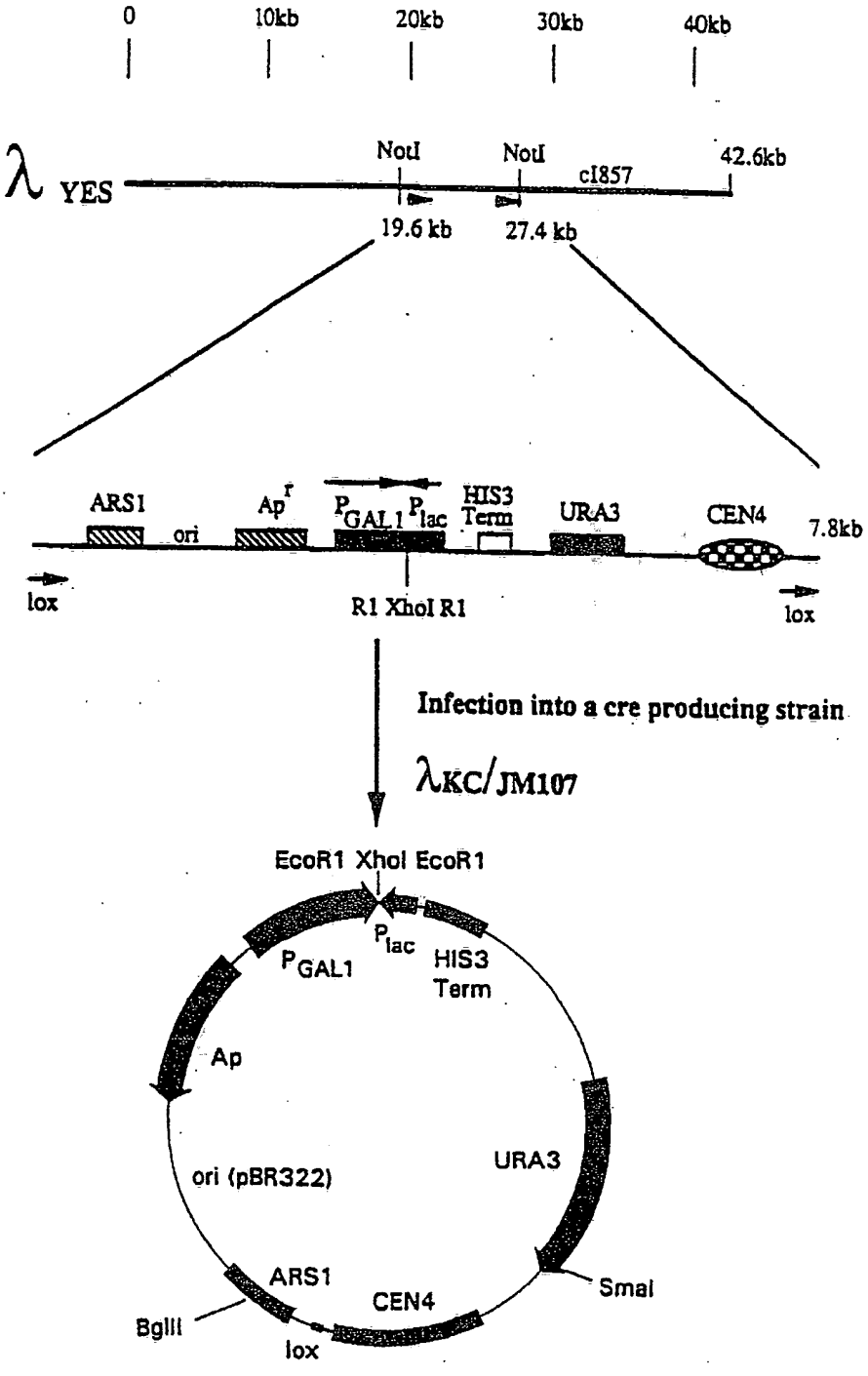


Fig. 4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NotI
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27.4 kb
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ori
Ap^r
P_GAL1 P_lac
HIS3 Term
URA3
CEN4
7.8kb
lox
R1 XhoI R1
lox
Infection into a cre producing strain
λKC/JM107
EcoR1 XhoI EcoR1
P_lac
P_GAL1
HIS3 Term
Ap
ori (pBR322)
URA3
SmaI
ARS1
CEN4
BglII
lox

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/03942

A. CLASSIFICATION OF SUBJECT MATTER
IPC(5) :C12N 1/18, 15/00, 15/29; C12Q 1/02; C12R 1:865
US CL :536/23.6; 435/7.31, 29, 172.3, 256, 320.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 536/23.6; 435/7.31, 29, 172.3, 256, 320.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
APS, DIALOG, SWISSPROT, PIR, EMBL, GENBANK, search terms: TRK1, TRK2, Saccharomyces, channel, sequence

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Genetics, Volume 125, issued June 1990, C.H. Ko et al., "TRK2 is Required for Low Affinity K^+ Transport in Saccharomyces cerevisiae", pages 305-312, see whole publication, especially page 309.	4-6,10-12,14-16,18 4-6,10-12,14-16,18
Y	WO, A, 92/02634 (Chandy et al.) 20 February 1992, see page 13 and 14, especially lines 23-28 of page 14.	4-6,10-12,14-16,18
A	Molecular and Cellular Biology, Volume 11, Number 8, issued August 1991, C.H. Ko et al., "TRK1 and TRK2 Encode Structurally Related K^+ Transporters in Saccharomyces cerevisiae", pages 4266-4273, see the abstract and column 2 of page 4266.	1-20

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be part of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
15 June 1993

Date of mailing of the international search report
04 AUG 1993

Name and mailing address of the ISA/US
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